and likely are part of novel cation channel family which is highly conserved from an evolutionary point of view; it shares homology with relevant proteins from C. *elegans* to humans. It is likely that αrENaC codes for the pore-forming subunit of one of the amiloride sensitive epithelial Na channels; however, until lipid bilayer reconstitution experiments are carried out one cannot rule out that the αrENaC codes for a regulatory protein that is intimately related to the Na channel. Studies have, however, demonstrated that similarly sized αrENaC transcripts are present in appropriate Na transporting tissues but are absent in tissues such as the liver and brain. Expression of αrENaC is developmentally regulated in the rat and human with the timing of the expression correlating with the lung's ability to transport Na. Expression of arENaC is increased by glucocorticosteroid hormones, which correlates with these hormone's ability to accelerate the maturation of Na transport in the intact developing lung.

The signal transduction pathways regulating Na transport have been extensively investigated in the kidney, and adult and perinatal alveolar epithelium. All three epithelia increase Na transport when intracellular [cAMP] increases; however the responsible cell membrane receptor is different. Although kidney epithelia can respond to AVP, V_2 membrane receptors are absent on perinatal alveolar epithelia, thus explaining why AVP does not increase alveolar epithelial Na transport. Similarly, the alveolar epithelium's Isc is unresponsive to aldosterone stimulation, and membrane permeant analogues of cGMP or PKC agonists do not increase its Na transport. In contrast, the alveolar epithelium increases [cAMP] and Na transport following β_2 receptor stimulation. The mechanism of action is complex and includes a β_2 agonist induced increase in intracellular [Ca], a nearly 2 log shift in the sensitivity of the NSC sensitivity to Ca, and a reduction in intracellular [Cl] which directly increases the open probability of the 25 pS amiloride-sensitive NSC in perinatal epithelium.

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Chloride transport inhibitors in epithelia

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Epithelial Cl transport involves the synchronized operation of Cl uptake systems, such as the Na/2Cl/K-cotransporter, and a Cl exit step, usually Cl channels. Cl transport can be inhibited at both sides of the epithelium. Loop diuretics such as furosemide, bumetanide and torasemide inhibit binding to the Na/2Cl/K-cotransporter by their high infinity. Cl channel blockers inhibit the Cl exit step. This mechanism of inhibition has been shown to be very effective in the thick ascending limb of the loop of Henle (TAL). However, the same compounds (NPPB) which inhibit Cl absorption in the TAL at <1 μmol/liter, have much less effect on the secretion of Cl in the colon. The reason for the different affinities of these blockers in various epithelia may be based on the differences of the respective Cl channels. Currently much evidence favors the view that the intermediate conductance outwardly rectifying Cl channel (ICOR) is not the key Cl exit mechanism in epithelia such as colonic crypt cells. Rather, it appears likely the small- or very-small-conductance Cl channels are responsible for epithelial Cl secretion. Unlike the ICOR-channel, these small channels are not very sensitive towards NPPB and related compounds.

In a broad search for putative inhibitors of Cl secretion we have also examined cromanol with the internal number 293 B. This compound proved to be a very potent inhibitor of Cl secretion in the colon. 293 B is a racemate. A separation of the two enantiomers 407 B and 434 B revealed that the latter compound is biologically active with an IC50 of only 250 nmol/liter from the serosal side.

In Ussing chamber experiments we examined whether 293 B is specific for cAMP-mediated Cl secretion. 293 B inhibited the equivalent short circuit current produced by forskolin, 8-CPT-cAMP, IBMX, VIP, PGE_2, adenosine, and cholera toxin. It was ineffective on the secretory Cl current produced by the Ca-ionophore, ionomycin. To examine the effect of 293 B in more detail, individual colonic crypts were perfused *in vitro* and the voltage across the basolateral membrane (V_{bl}) was measured. It was shown that agonists acting via cAMP induce a rapid but transient depolarization followed by a sustained, less pronounced depolarization. During this phase the membrane conductance of the impaled cell was maximal. 293 B added to the basolateral side depolarized V_{bl} further by 6 to 10 mV. This suggests that 293 B reduced a K conductance. However, 293 B had no effect on the basal K conductance, nor on the K conductance induced by ATP. Comparable whole cell patch clamp studies in CFPAC-1 cells, which show little or no Cl current in response to cAMP, have revealed that 293 B also inhibited a cAMP-induced K conductance. The present data indicate that Cl transport can be inhibited reversibly by a new class of inhibitors of the cromanol type (293 B). These substances apparently inhibit a K conductance which is activated by cAMP. Inhibition of this conductance then limits the amount of Cl secreted.

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Solute and fluid secretion mechanisms in ADPKD cells

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Fluid secretion is a major factor involved in renal cyst growth in patients with autosomal dominant polycystic kidney disease. We have begun an investigation into the nature of the solute transport systems that drive that secretion. The data we have collected thus far are compatible with the hypothesis that chloride secretion drives fluid transport by the ADPKD cystic tissue. Three preparations were used in this investigation; all are derived from kidneys removed from ADPKD patients. First, intact cysts were dissected from these kidneys and their ability to secrete fluid was measured. Second, monolayers of cells of primary cultures of cystic tissue were grown on permeable supports. Their ability to secrete fluid and their electrical properties were determined. Third, microcysts were formed by clonal growth of cells from primary cultures of cystic tissue. Single cysts were isolated and video and epifluorometric techniques were used to measure fluid secretion, cell volume and changes in cell potential and cell chloride concentration.

Individual superficial cysts were dissected from ADPKD kidneys, the cyst fluid was removed and replaced with culture medium, and a gravimetric technique was used to measure fluid loss or gain by the cyst in 24 hour periods. A series of experiments was performed to test the effect of the adenylate cyclase agonist, forskolin, and ouabain on fluid transport by the isolated cysts. Forskolin caused the cysts to secrete fluid. Ouabain, added to the cyst cavity (apical surface) in the presence of forskolin, did not affect secretion. Addition of the inhibitor to the bath (basolateral surface) completely blocked fluid secretion. The results of these experiments indicate that the location of functional Na/K-ATPase is on the basolateral surface of these cysts.

Primary cultures were grown from the inner walls of ADPKD cysts and confluent cultures of the resulting cells were grown on the permeable membrane of Transwell-Col culture chambers. Forskolin induced fluid secretion by these monolayers. The monolayers of these cells and the supporting membrane were also mounted in standard Ussing chambers and the transepithelial potential difference, V_{te}, the short-circuit current, SCC, and tissue resistance were measured. In 36 monolayers, the apical surface was negative with respect to the basolateral surface and V_{te} was hyperpolarized by the addition of forskolin. SCC measurements indicated a positive ionic current flowed from the apical to the basolateral surface and this current was increased by forskolin. The transepithelial resistance averaged 156 ohms $\cdot$ cm^2 and was reduced by forskolin. These measurements are not compatible with the thesis that the cystic tissue secretes Na. The effect of ouabain on the forskolin-treated monolayers was also tested. Apical application of ouabain did not affect V_{te} or SCC. However, basolateral application of ouabain depolarized the monolayers and reduced SCC nearly to zero. These data indicate that the location of functional Na-K, ATPase is on the basolateral surface of monolayers of cultured ADPKD tissue.

Since mislocation of Na/K-ATPase to the apical membrane and sodium secretion could not account for fluid secretion in intact and in cultured ADPKD tissue, we considered that secretion of chloride may drive fluid secretion much as it does in other secretory epithelia. We hypothesized that chloride enters the cell across the basolateral membrane via a Na-K-2Cl cotransporter in the basolateral membrane and exits the cell via a conductance pathway in the apical membrane activated by cAMP. We began our investigation of this hypothesis by applying the chloride channel blocker, diphenylamine-2-carboxylate, DPC, to the apical membrane of forskolin-treated monolayers. DPC depolarized the tissue and greatly decreased the SCC. These data suggest that a chloride channel in the apical membrane may be involved in fluid secretion. However, much more work is needed to verify that tentative conclusion. In the second series of experiments we examined the effects of bumetanide, an inhibitor of the Na-K-2Cl cotransporter. Bumetanide inhibited the forskolin-induced increase in SCC and depolarized V_{te}. These results are compatible with the hypothesis that a Na-K-2Cl cotransporter is present in the basolateral membrane of cystic tissue and participates in the fluid secretion initiated by forskolin.

Microcysts grown from single, cultured ADPKD cells seeded in a collagen matrix were isolated and placed in a superfusion chamber on the stage of an inverted microscope. Morphometric techniques were used to measure the rate of change in cavity volume and the volume of the cells forming the cyst. Epifluorimetric techniques were used to measure the changes in cell electrical potential as reported by changes in the fluorescence of bisoxonol, a lipophilic anion. Fluid secretion into the cystic cavity was induced by the application of 8-bromo-cAMP. The initiation of secretion was accompanied by a loss of cell volume and hyperpolarization of the cell potential. Bumetanide blocked the secretion, caused a further loss in cell volume and an additional hyperpolarization. In preliminary experiments, changes in cell chloride concentration were determined with the use of the fluorescent indicator, methoxy-ethyl quinolinium (MEQ). The application of bumetanide to a microcyst, stimulated to secrete fluid by cAMP, caused a sharp reduction in cell chloride concentration that was reversed when the inhibitor was removed. The results obtained with the microcysts were interpreted to support the hypothesis that chloride secretion drives fluid secretion by cystic tissue as it does in airway epithelia and in certain cultures of intestinal epithelia.

The data reported here did not confirm the thesis that mislocation of Na/K-ATPase to the apical membrane and the secretion of sodium is responsible for fluid secretion by ADPKD cysts. The data did indicate that an anion is secreted by ADPKD tissue and are compatible with the hypothesis that fluid secretion is driven by chloride secretion.

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